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STUDIES ON THE PLANT CELL.—II.

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THE ACTIVITIES OF THE PLANT CELL.

I. Vegetative Activities.

EVERY cell passes through a history whose events repeat in a broad way activities that have become established in the organism by the experience of its ancestors. The most important of these events is nuclear division, which is accompanied in most plants by cell division, the important exceptions being certain groups whose protoplasm is multinucleate throughout all, or almost all, vegetative conditions (*e. g.*, cœnocytic Algæ and Fungi, plasmodia and multinucleate cells in various tissues).

Protoplasm, whose nuclei can no longer divide, becomes incapable of reproducing itself and must take a dependent position in the organism, where the length of its life will be determined by the good fortune of its environment and its vitality. Such protoplasm becomes strictly vegetative in its functions, and while these activities may be very highly specialized and of the utmost importance to the organism as a whole, nevertheless such a cell has lost certain of the constructive, and in consequence reproductive, possibilities characteristic of living matter. The most evident and important of these constructive activities have to do with the increase of nuclear material (chiefly chromatin), which leads to its distribution through nuclear division, and the development of a complicated mechanism (the spindle) to effect this result.

As Weismann first pointed out, from the standpoint of cell studies, there is a stream of germ plasm flowing with every species, protoplasm relatively fixed in its characteristics and potentially immortal. The chief peculiarities of germ plasm are its reproductive powers and the generalized structure that enables it

to turn to any form of activity possible to the species. Portions of the germ plasm are constantly being detached from the main stream and relegated to more or less special duties. Such protoplasm becomes the body plasm, or soma, of the individual. Specialized body plasm generally loses very shortly the reproductive possibilities of germ plasm, and in consequence must finally die, for its nicely adjusted dependence upon surrounding cells cannot last forever. Yet it has been one of the surprises of biological science that specialized tissues may keep for a very long time the reproductive qualities of germ plasm. Investigations on regeneration in particular have brought these facts conspicuously to the front. As an extreme example among plants, it is known that even the epidermal tissues of leaves and scales of certain ferns (Palisa, :00) may sometimes retain the fundamental qualities of germ plasm and reproduce the plant.

There are no visible characters that separate body plasm from germ plasm, excepting, of course, when body plasm begins to show signs of degeneration. Germ plasm may only be distinguished by its potentialities of growth and reproduction, potentialities that cannot be accurately determined because the stimulus to development is, in the last analysis, an external one and the conditions which govern it may be so intricate as to escape close scrutiny.

Germ plasm is found in its most generalized condition in the cells of growing points, in embryonic and meristematic regions, and in the reproductive tissues. These tissues are well recognized as the most favorable for cell studies because they present most clearly the details of protoplasmic activities. Almost all that we know of cell activities have come from investigations of such regions.

One of the first signs of that specialization which transforms germ plasm to body plasm is the slowing up and final end of nuclear and cell division. With this change come a great variety of modifications (peculiarities of cell wall, plastids, cytoplasmic activities, etc.) which may be readily associated with the particular work of that tissue.

The vegetative activities of germ plasm are chiefly those of growth, which in the end mean reproduction, the embryonic cells

drawing upon food that has been prepared for them and is either stored in special structures (as seeds, spores, bulbs, etc.), or manufactured in differentiated organs or tissues (leaves, chlorophyll bearing tissue, phloem, etc.). The vegetative activities of body plasm are far more specific than those of germ plasm. Their tissues have particular and highly developed activities, some dealing chiefly with photosynthetic processes, some (phloem) distributing the organized food over the plant body, some storing the food in large quantities. Besides these there are mechanical functions performed by highly differentiated tissues, even though largely composed of empty cells, as the vascular tissue, supporting tissues, and the external protective integuments.

It is not our purpose to discuss any of these vegetative activities in detail, but only to distinguish as sharply as possible the characteristics of germ plasm with its generalized activities from the specialized body plasm. These generalized characters, as before stated, are constructive activities which mean growth and lead to nuclear and cell division. It is probable that any tissue which presents them has regenerative powers that under the proper environment might be expected to reproduce parts or the entire organism. Germ plasm is distributed more widely throughout the organism than is generally supposed, and many highly specialized tissues still retain the spark of regenerative possibilities. The significance of these conditions is not generally appreciated, perhaps because the environmental conditions of regeneration are little understood and are exceedingly hard to adjust experimentally. There is presented here a very attractive field of botanical investigation, a union of cell studies with the more gross anatomical methods of experimental morphology.

2. Cell Division.

Cell division takes place only after periods of growth that have led to a multiplication of nuclei and in the tissues of plants above the thallophytes is very generally a part of the history of each mitosis. This is because of the structure called the cell plate which is essentially an organ of cell division. But the thallophytes present other methods of cell division which bear no especial relation to nuclear activities, and in certain groups of

the thallophytes nuclear division may proceed through the entire vegetative life of the organism without any segmentation of the protoplasm which only takes place during the reproductive phase of spore formation. But fundamentally protoplasmic segmentation depends on increase in the amount of protoplasm which demands the multiplication of nuclei so that nuclear division always precedes cell division, and we shall consider the events in that order.

(a) Events of Nuclear Division.

1. Direct Division.

The nucleus divides after one or two methods, either directly by constriction or fragmentation, or indirectly (mitosis) when there is present a fibrillar apparatus called the spindle. Direct division is the only form present in the simplest plants and phylogenetically must have preceded the elaborate mechanism demanded for indirect division. This topic will be given especial attention in Section VI. Direct division is also present in certain specialized cells and tissues of higher plants. These are generally old cells or tissues that are far removed from the generalized structure and potentialities of germ plasm. Yet sometimes direct and indirect division occur in the same cell, *e. g.*, *Valonia* (Fairchild, '94), and such forms might be made the subject of very interesting investigations. In some cases the phenomenon of direct nuclear division accompanies pathological conditions or the degeneration of cells and may take the form of extensive fragmentation. It would be outside of our purpose to discuss such phenomena which is obviously abnormal, and the primitive forms of nuclear division will be taken up later (Section VI). It is possible that direct division in higher plants is in a sense a reversion to early ancestral conditions, a reversion that only comes on when for some reason the normal activities of the germ cell are in abeyance or have ceased.

2. Indirect Division (Mitosis).

Indirect nuclear division, mitosis or karyokinesis, is characterized by a mechanism which varies greatly among plants in its

method of development. The characteristic appearance of this apparatus is a spindle like figure formed of fibrillæ. The poles of the spindle may be occupied by centrosomes or centrospheres or they may be entirely free from such organized kinoplasmic bodies. The essential structures of the spindle are sets of contracting fibers which separate the chromosomes into two groups drawing them to the poles of the spindle where the daughter nuclei are organized. But besides these fibers there are generally present other fibrillæ which complicate the nuclear figure. Some of these extend from pole to pole (spindle fibers) others lie outside of the spindle and end freely in the cytoplasm or attach themselves to chromosomes (mantle fibers), and if centrosomes or centrospheres be present there are likely to be fibers radiating from these centers to form asters.

The events of mitosis are generally grouped into four periods : (a) Prophase, to include the formation of the spindle and preparation of the chromosomes ; (b) Metaphase, the separation of the daughter chromosomes ; (c) Anaphase, the gathering of the daughter chromosomes into two groups which pass to the poles of the spindle ; (d) Telophase, the organization of the daughter nuclei. It is almost needless to say that these periods merge so gradually one into the other that sharp lines cannot be drawn between them. The activities during prophase are especially variable.

Prophase. — There are two types of spindles in plants, (1) those that are formed within the nuclear membrane and (2) those whose fibers originate largely or wholly from kinoplasm outside of the nucleus. Intranuclear spindles have been reported in a number of groups of the thallophytes. They seem to be the rule in the mitoses of oögenesis in the Peronosporales (Wager, '96, :00, Stevens, '99, :01 and :02, Davis, :00, Miyake, :01, Trow, :01, Rosenberg, :03). They are present in Saprolegnia, Fig. 5a (Davis, :03). Fairchild ('94) reports them for Valonia. Farmer and Williams ('98, p. 625) state that the spindle of *Ascophyllum* is largely intranuclear. Harper (:00) has not described them for the Myxomycetes, but very little is known about the prophases of mitosis in that group and their presence is quite probable. Timberlake (:02) is not positive whether the

spindles of *Hydrodictyon* are intranuclear or not; they lie in a clear space which, however, may be a vacuole rather than the outline of a nuclear cavity. It seems probable in such a type that the vacuole is really the nuclear cavity whose plasma membrane (nuclear membrane) becomes less clearly defined. The development of the spindle is very difficult to follow among these lower forms because it is so small. Stevens (:03) found an exceptionally favorable type in *Synchytrium* and came to the conclusion that the spindle developed from the threads of the spirem (linin) entirely within and independent of the nuclear membrane.

Very remarkable intranuclear spindles have been described in the central cell of the pollen tube of *Cycas* (Ikeno, '98 b) and *Zamia*, Fig. 5d (Webber, :01). Murrill (:00) found them in the mitosis following the fusion of gamete-nuclei in the egg of *Tsuga*, Ferguson (:01b) at the same period for pine, and Coker (:03) in *Taxodium*. They are also reported by Strasburger (:00) in the cells of young anthers and nucelli of the lily and in growing points (*Viscum*) and possibly may be found quite generally in cells weak in kinoplasmic cytoplasm. The development of the spindles in the above forms has not been studied in detail, but the fibers are probably derived from the linin. We are given a clue to the process by the events of spindle formation in the spore mother cell of *Passiflora* (Williams, '99). In this angiosperm the nuclear cavity becomes filled with a fibrillar network developed from the linin, the nuclear wall becomes transformed into a mesh connecting the intranuclear fibers with a surrounding cytoplasmic reticulum. The fibers in the central region of this net work develop the spindle which is consequently very largely of intranuclear origin.

Among the thallophytes the poles of intranuclear spindles are frequently occupied by deeply staining bodies which have been called centrosomes; but these structures can hardly be homologous with the well-known centrosomes of other thallophytes, *e. g.*, *Stypocaulon* (Swingle, '97) and *Dictyota* (Mottier, :00). They are probably merely temporary accumulations of material with no morphological significance.

Spindles that arise from fibers external to the nucleus (extra

nuclear spindles) are of two main types: (1) those associated with centrosomes, centrospheres or kinoplasmic caps, and (2) those composed of independent fibrillæ developed as a mesh around the nucleus. The latter condition is especially characteristic of the spore mother cell and is perhaps the highest type of spindle formation known for either animals or plants. It is very interesting to trace the relations of this highest condition to the lower types through certain lines of evolution to be discussed in Section VI.

Spindles with centrosomes are known in *Sphacelaria*, *Stypocaulon* (Swingle, '97), *Dictyota*, Fig. 4 *a* (Mottier, :00), the zoö-sporangium of *Hydrodictyon* (Timberlake, :02), in certain diatoms (Lauterborn, principal paper '96, Karsten, :00) and in the basidium (Wager, '94 and Maire, :02). The best accounts of the behavior of the centrosomes are given by Swingle and Mottier. Indeed there is much doubt about the history and significance of the bodies in the other forms, although the constancy of their presence at the poles of the spindles indicates that they are really centrosomes. The conditions in the diatoms are especially complicated; an account of Lauterborn's work has been published in English by Rowley, :03. In *Stypocaulon*, *Sphacelaria* (Fig. 3 *c*, Section I) and *Dictyota* (Fig. 4 *a*) the cells studied have permanent asters which lie at the side of the nucleus and which divide just previous to the mitosis and separate so that they come to lie on opposite sides of the nucleus. Fibers develop from the centrosomes on the sides nearest the nucleus and elongating push against the nuclear membrane and finally enter the nuclear cavity to form the spindle.

Spindles with centrospheres are well known in *Fucus* (Farmer and Williams, '96, '98, Strasburger, '97a), *Corallina*, Fig. 5 *c*, (Davis, '98), in the ascus, Fig. 5 *b* (Harper, '97 and '99), and in the germinating spore of *Pellia*, Fig. 4 *c* (Farmer and Reeves, '94, Davis, :01, Chamberlain, :03). Centrospheres have been reported in other forms but the types mentioned above have received the most careful study. It is probable that the centrosphere is but a larger, more generalized kinoplasmic center than the centrosome, a protoplasmic region whose dynamic activities do not focus so sharply as in the latter structure. There are

bodies, as in the basidium, which stand intermediate in size between centrosomes and centrospheres and are probably only called the former because they are very distinct in outline.

Centrospheres in *Fucus* (Fig. 3 *d*, Section I), *Corallina* (Fig. 4 *b*) and *Pellia* (Fig. 3 *e*, Section I, Fig. 4 *c*) are formed *de novo* for each mitosis by an accumulation of kinoplasm at the poles of the elongating nucleus. The centrospheres in the ascus divide before each of the three successive mitoses and finally remain, one for each nucleus, to instigate the peculiar process of free cell formation characteristic of the ascus. Centrospheres are frequently the centers of asters which, however, are usually not as sharply defined as those with centrosomes, possibly because the fibers are not grouped with the same degree of symmetry as is shown around centrosomes.

Spindle fibers from centrospheres develop in precisely the same manner as from centrosomes, *i. e.* by the growth of the fibrillæ into the nuclear cavity through the dissolving nuclear membrane. The activity is well shown in the oögonium of *Fucus*, and Farmer ('98, p. 638) believes "that the intranuclear part of the spindle is differentiated out of nuclear material that is unused for chromosome formation." The entrance of spindle fibers from centrospheres at the ends of a nucleus has been observed by myself in *Corallina*, Fig. 4 *b* (Davis, '98). The germinating spores of *Pellia*, Fig. 4 *c* (Davis, :01, Chamberlain, :03) furnish especially good illustrations of the entrance of spindle fibers into the nuclear cavity and the development of the spindle in this form is coincident with the dissolution of the nucleus which, according to Strasburger's theory ('95), indicates that the latter structure contributes material for the growth of spindle fibers.

In connection with the centrosphere mention should be made of the blepharoplasts of the cycads and Ginko which are remarkable bodies with radiating fibers. They have been considered by some as asters with centrosomes, but it is known that they take no part in spindle formation or other mitotic phenomena in these forms, and consequently need not be considered at this time. They will be treated in some detail in the account of the sperm (Section III).

Kinoplasmic caps which form spindles are probably an evolution from the type of centrosphere that is developed *de novo* with each mitosis as in *Pellia*. Such centrospheres by becoming less definite in form and lacking radiating fibers would be called kinoplasmic caps. Indeed the centrosphere so evident in the early cell divisions of the germinating spore of *Pellia* becomes a kinoplasmic cap in the later mitoses of the older gametophyte (Davis, :01).

Spindles developed from kinoplasmic caps are characteristic of

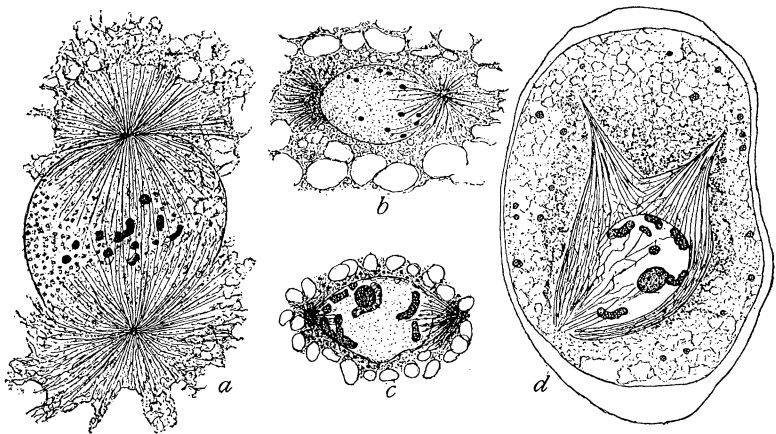


FIG. 4.—Prophases of Mitosis. *a*, *Dictyota*; late prophase in spore mother cell, fibers from the two asters with centrosomes have entered nuclear cavity to organize the spindle, chromosomes gathering to form the nuclear plate. *b*, *Corallina*, early prophase in tetra spore mother cell; two centrospheres, the fibers from one having entered the nuclear cavity, chromosomes shown. *c*, *Pellia*, nucleus in germinating spore; spindle fibers from ill defined centrospheres entering nuclear cavity, chromosomes and a nucleolus present. *d*, *Gladiolus*, first mitosis in pollen mother cell; a multipolar spindle, nuclear wall breaking down at one side and fibrillae entering the nuclear cavity, chromosomes and a nucleolus present. After Mottier and Lawson.

the mitoses in vegetative tissues, meristematic and other embryonic regions. They have been especially studied in higher plants by several investigators and for a large number of forms, those most completely described being *Psilotum* (Rosen, '95), *Equisetum*, *Allium* and *Solanum* (Nemec, '98a and '98b, '99b and '99c), *Pteris*, *Ephedra* and *Vicia*, (Fig. 3 *f*, Section I) (Hof, '98) and *Allium* (McComb, :00). The polar caps first appear as accumulations of kinoplasm on opposite sides of the nucleus which generally elongates. The protoplasm is granular and although

central bodies have been reported most investigators are agreed that they are only granules without regularity or special significance. They are no longer believed to be centrosomes. Fibrillæ are developed from the kinoplasmic caps and grow out against the nuclear membrane and finally enter the nuclear cavity to form the spindle. A large part of the substance of the kinoplasmic cap is transformed into these spindle fibers.

Papers by Schaffner ('98) on *Allium* and Fulmer ('98) on the seedling of the pine are the last attempts to bring the centrosome into the history of spindle formation in vegetative tissues of higher plants. But their results cannot stand against the accumulation of studies which indicate that centrosomes are not present in the cells of any plant above the thallophytes with the possible exception of the mysterious blepharoplast and certain structures appearing in some phases in the life history of *Hepaticæ*. Centrospheres are unquestionably present in the *Hepaticæ* and centrosomes have also been reported. The centrospheres are, however, so generalized as to approach the kinoplasmic caps in structure and development and it seems quite possible that they are the forerunners of this manifestation of kinoplasm. The so-called centrosomes of the liverworts do not exhibit the specialized structure or behavior of centrosomes among the thallophytes and it is probable that they are only smaller and somewhat more clearly defined centrospheres. These structures in the *Hepaticæ* seem to hold an intermediate relation between the definite kinoplasmic bodies (asters, centrosomes and centrospheres) of the thallophytes and the remarkable kinoplasmic activities in higher plants which reach their highest expression in the processes of spindle formation in the spore mother cell. These topics will be treated in Section VI.

Structures resembling kinoplasmic caps have been reported in several other tissues than those noted above. Thus Murrill (:00) finds in the formation of the ventral canal cell of *Tsuga* a dense fibrous accumulation beneath the nucleus which develops one pole of the spindle in essentially the same manner as other polar caps. The other pole of the spindle in this case appears to be formed differently for the fibers seem to be intranuclear.

It would be interesting if two types of spindle formation were present at opposite poles of the same nucleus and further investigation of this subject is much to be desired. The mitoses in the central cell of *Pinus* (Ferguson, :01b, Chamberlain, '99, and Blackman, '98) and *Picea* (Miyake :03a) show spindle formation from accumulations of fibrillæ outside of the nucleus but without conspicuous polar caps. Still more striking than the irregular spindle of Murrill in *Tsuga*, described above, is Miss Ferguson's (:01a) account of the mitosis in the generative cell of the pollen grain of *Pinus*. The spindle here begins to develop as a cap-like accumulation of kinoplasm below the nucleus. The fibers enter the nuclear cavity and in coöperation with a nuclear reticulum form a system of fibers that extend through the nuclear cavity to the inner side of the nuclear membrane beyond. This portion of the nuclear membrane persists until after metaphase so that one pole of the spindle is found wholly within the nucleus while the other is external and of unquestioned cytoplasmic origin. Coker, :03, regards the spindle which differentiates the nucleus of the ventral canal cell in *Taxodium* as almost wholly of nuclear origin and the chromosomes as derived largely from the nucleolus. There are evidently some interesting complications in this form which deserve further study.

It should be noted that whenever spindles are formed in connection with centrosomes, centrospheres or kinoplasmic caps that the fibers have a definite region of attachment from which they extend into the nuclear cavity. Such regions constitute a sort of anchorage for the spindle fibers. In this respect the physiological side of the process of spindle formation in these forms is quite similar to that of the animal kingdom and in sharp contrast to other methods that are found in higher plants, which will now be considered.

When spindles are formed after the second method, *i. e.*, by independent fibrillæ making up a network around the nucleus, there is an abrupt change in the method of development. The kinoplasm becomes distributed around the nucleus as an investing layer and shows no inclination to gather into centers such as kinoplasmic caps or centrospheres. There is developed

from this granular kinoplasm a meshwork of fibrillæ that extends into the cytoplasm more or less radially. When the nuclear membrane becomes disorganized the fibers enter the nuclear cavity and organize the spindle (see Fig. 4 *d*). In some forms, *e. g.*, *Passiflora* (Williams, :00), many or most of the fibers are developed in the interior of the nucleus from the linin and become connected with the extra nuclear reticulum by the dissolution of the nuclear membrane.

The free ends of the fibrillæ that lie in the cytoplasm become gathered into several poles which are distributed variously around the nucleus. This condition constitutes the so-called multipolar spindle (Fig. 3 *g*, Fig. 4 *d*), which in its highest type of development illustrates the most complex method of spindle formation known for animals or plants. During the later periods of prophase the several poles of the multipolar spindle converge and fuse with one another into two poles with a common axis, thus forming the mature bipolar spindle of metaphase (Fig. 5 *f*). The spindle is in a broad sense bipolar, but one may readily see that each pole is made up of several groups of fibrillæ which generally remain quite independent of one another (Fig. 5 *f* 1).

The relation of a multipolar stage to the bipolar spindle of metaphase was first made clear by Belajeff ('94b) for *Larix*, and later was established more widely by the investigations of Osterhout ('97) on *Equisetum*, Mottier ('97) for the lily, and Juel ('97) for *Hemerocallis*. This type of spindle formation is now well known in the spore mother cells of numerous spermatophytes and several pteridophytes. The same conditions in simpler form are found in the spore mother cells of the Hepaticæ, *e. g.*, *Anthoceros* (Davis, '99), *Pellia*, Fig. 5 *e* (Davis, :01), and *Pallavicinia* (Moore, :03). There are a number of very interesting peculiarities in this type of spindle which presents a wide range of variation in the details of its fibrillar organization and development. These will receive special treatment in the account of the spore mother cell (Section III).

The only types of thallophytes known, in which the spindle is partly or wholly of cytoplasmic origin without centrosomes, centrospheres, or kinoplasmic caps, are *Chara* (Debski, '97) and *Spirogyra* (Van Wisselingh, :02). The developmental history is

very difficult to follow in these forms and is not fully known, but multipolar conditions are reported which later change into bipolar spindles.

While the spindle is being organized by kinoplasmic activities outside of the nucleus, some events occur within which form a very important part of the prophases of mitosis. The linin material, which in the resting nucleus generally has the form of a net, becomes organized into a much looped ribbon, called the spirem thread. The chromatin material gathers along the spirem thread as deeply staining globular bodies. These split into halves in the direction parallel with the axis of the spirem thread, and the two sets of chromatic bodies lie in two rows along the edge of the ribbon, which shortens as it grows older. Finally the spirem thread divides transversely into a definite number of segments, and these are the chromosomes. The chromosomes are generally fully formed at the time when the spindle fibers enter the nuclear cavity, and they are readily moved as the fibrillæ develop the spindle. Some of the fibers become attached to the chromosomes, carrying them to the equatorial region of the spindle to form the structure called the nuclear plate, which always indicates the approach of metaphase.

As the spirem thread matures the amount of chromatin is greatly increased, so that the separate globules run together and cannot be distinguished in the chromosome which is homogeneous in structure. Chromatin has its greatest staining power at this period. Whether linin is closely related to chromatin in composition and is actually changed into that substance, or whether it dissolves and contributes its material to the growth of the spindle, is a problem of some importance as yet unsolved. It is possible that the nucleolus may furnish material for the chromosomes, and some nucleolar like bodies are known to be chromatic in character, but it does not seem to be established that any of these are genetically related to an unquestioned nucleolus in any plant form.

Important changes come over the nucleolus coincident with the development of the chromosomes. The structure frequently gives signs of internal modifications early in prophase and before the development of the spindle. It may gradually fade away or

decrease in size, or, if large, it may fragment. Strasburger, in 1895, advanced the view that the spindle actually drew upon the substance of the nucleolus for the material and energy necessary to its development. The evidence in support of this suggestive theory lies chiefly in the development of the spindle coincident with the dissolution of the nucleolus. There is also some evidence that the nucleolus contributes material to the developing chromosomes. Small globules, which stain as the substance of the nucleolus, may sometimes be found adhering to the chromosomes as though becoming incorporated in them. These subjects are naturally very difficult of investigation because stain reactions cannot be depended upon with certainty and are not, of course, chemical tests. Then the behavior of the nucleolus during mitosis is exceedingly variable, since it sometimes disappears quickly and sometimes remains intact, and it becomes a very difficult matter to determine its importance. The nucleolus is probably not absolutely necessary at any stage in mitosis, for both spindle fibers and chromosomes develop apart from this structure; but it does seem to be established that the substance of the nucleolus is generally drawn upon by the cell, especially during prophase, when numerous spindle fibers are organized and the amount of chromatin is being largely increased. Experiments of Hottes, which unfortunately have never been published, have an important bearing on these problems.

If the nucleoli are not entirely dissolved they are frequently thrown out of the spindle into the cytoplasm, where they may lie for long periods as deeply staining globules which are sometimes called extra nuclear nucleoli. It is probable that very many of the bodies that pass under this cumbersome title have no relation whatever to the nucleolus. The cytoplasm frequently contains globules that may be coagulated or precipitated food products, and all of these stain similarly to nucleoli.

Metaphase. — The period of mitosis termed metaphase is, to speak precisely, the time when the two halves of the split chromosome separate from one another. However, this is a period of such short duration that for practical purposes nuclei are considered in metaphase when their chromosomes are lined up at the nuclear plate. The metaphase of mitosis is generally the most

conspicuous of the nuclear activities not only on account of the position of the chromosomes (see Fig. 5), but because all ktoplasmic structures (the fibrillæ and centrosomes or centrospheres, if present) are shown to their best advantage.

The best evidence indicates that the chromosomes of plants

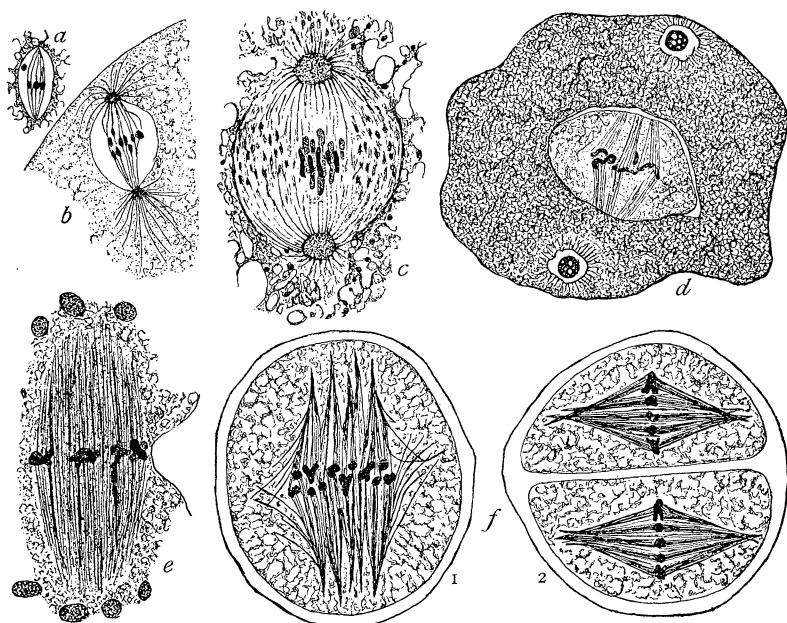


FIG. 5.—Metaphases of Mitosis. *a*, *Saprolegnia*; intranuclear spindle in oögonium, nucleolus outside of spindle. *b*, *Erysiphe*; mitosis in ascus, asters with rather small centrospheres. *c*, *Corallina*; first mitosis in tetraspore mother-cell, very large and well differentiated centrospheres. *d*, *Zamia*; blunt poled intranuclear spindle in central cell of pollen grain; blepharoplasts, their outer membrane about to break up. *e*, *Pellia*; first mitosis in spore mother-cell; broad spindle with rounded poles, the very numerous spindle fibers ending in granular ktoplasm. *f*, *Agave*; first and second mitoses in pollen mother-cells; (1), multipolar spindle just previous to metaphase; the several independent cones of fibrillæ gather more closely together to complete the spindle. (2) metaphase of second mitoses; completed spindles showing however the several independent cones of fibrillæ. (After Harper, Webber and Osterhout.)

only divide longitudinally. This matter has considerable theoretical interest, which will be considered in Section V, and also in connection with the spore mother cell (Section III). The daughter chromosomes are drawn apart by the contraction of the fibrillæ to which they are attached.

Chromosomes may take on various forms during metaphase,

especially while they are being separated. Thus, if the chromosomes are dragged apart from the ends, their form is generally rod shaped; but if the attachment of the fibrillæ is near the middle of the chromosome, the structures are pulled apart as loops or V's, and the pair of chromosomes just previous to their separation may be ring shaped. A further complication is introduced in the spore mother cell by certain premature divisions by which each daughter chromosome becomes a pair of granddaughter chromosomes instead of remaining a single structure. The peculiarities of the heterotypic and homotypic mitoses are due

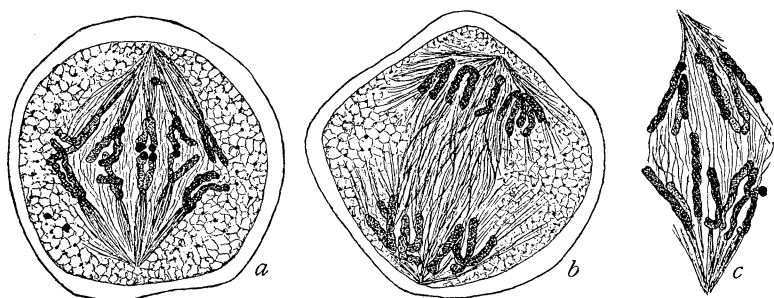


FIG. 6.—Anaphase of Mitosis, spore mother-cell of *Lilium martagon*. *a*, immediately after metaphase of first mitosis; each daughter chromosome consists of two grand daughter segments, adhering at the ends, making the familiar V-shaped figures characteristic of the first mitosis (heterotypic) in the spore mother-cell of higher plants. *b*, late anaphase of the first mitosis; the V shaped chromosomes, each composed of two grand daughter segments adhering at the ends, are very close to the poles of the spindle; the central fibers of the spindle are conspicuous at this stage. *c*, second mitosis; the grand daughter chromosomes, that composed the Vs of the first mitosis, have separated at the nuclear plate of the second mitosis and are being drawn by their ends to the poles of the spindle where they will organize the nuclei of the pollen grain; this mitosis is called homotypic to distinguish it from the usual (typical mitoses in which there are no premature divisions of the chromosomes. All figures after Mottier.

to this phenomenon. (See account of spore mother cell in Section III.

Anaphase.—Anaphase begins with the separation of the daughter chromosomes at the nuclear plate (Fig. 6) and ends with the gathering of these structures at the poles of the spindle preparatory to the organization of the daughter nuclei. As the chromosomes move towards the poles the fibers of the central spindle stand out sharply (see Fig. 6 *b*). If a cell wall is to be formed between the daughter nuclei one may expect to find these fibers thickening in the equatorial region of the

spindle where the nuclear plate formerly lay. Such thickenings are granular accumulations formed by the contraction of the central spindle fibers and mark the beginnings of the cell plate (Fig. 8 *d*) that afterwards gives rise to the cell wall. When the daughter chromosomes reach the poles of the spindle they generally lie in a region of granular kinoplasm which results in part from the contraction of spindle fibers and in some cases from the breaking down of organized centrospheres (*c. g.*, *Coralina*, *Pellia*, *Fucus*, etc.). The daughter nucleus at this time

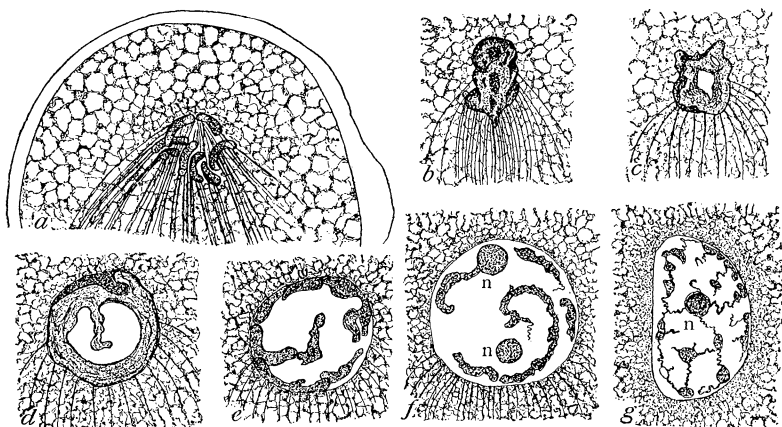


FIG. 7.—Telophase of Mitosis, spore mother-cell of *Passiflora carulea*. *a*, very late anaphase; the daughter chromosomes are collected at the poles of the spindle. *b*, the commencement of telophase; the chromosomes have fused together and the daughter nucleus is represented by an irregularly shaped mass of chromatin. *c*, the presence of small lacunæ within the mass of chromatin indicates the accumulation of nuclear sap in vacuoles. *d*, an increased amount of nuclear sap, still held however within the mass of chromatin, and consequent enlargement of the vacuole destined to become the nuclear cavity. *e*, the chromatin has begun to break up into small masses so that it no longer holds the nuclear sap which has established contact with the cytoplasm and is forming the nuclear plasma membrane. *f*, nuclear sap in contact on all sides with the cytoplasm and a complete nuclear membrane clearly established; chromatin is very much broken up and two nucleoli (*n*) have been formed. *g*, the resting nucleus with chromatin distributed in small masses connected by a network of linin threads; a nucleolus (*n*) is shown; the zone outside the nuclear membrane is kinoplasm and its appearance indicates the approach of the second mitosis in the pollen mother-cell. All figures after Lawson.

(Fig. 7 *a*, *b*) is in its simplest terms, as explained in Section I, a group of chromosomes surrounded by granular kinoplasm and without the nucleolus, linin network or the vacuole which later contains the nuclear sap.

Telophase.—Telophase is the closing period of mitosis and completes the organization of the daughter nuclei (see Fig. 7).

The chromosomes come to lie in a vacuole (Fig. 7 *c, d, e*) containing nuclear sap and later the chromatin becomes distributed over a linin network and one or more nucleoli develop (Fig. 7 *f, g*). As was stated in Section I, the nuclear membrane probably represents the reaction of the granular kinoplasm to a fluid secretion around the chromosomes which becomes the nuclear sap (Lawson, :03a). However, the nuclear membrane is generally a definitely organized film, much more sharply defined than vacuolar membranes. The development of the linin network is not well understood. It is readily seen that the chromosomes become joined end to end and sometimes elongate. The amount of chromatin diminishes as the linin substance appears, but it is not certain whether the chromatin is changed directly into linin, or whether the latter substance is a secretion. The best evidence rather favors the former view. Nucleoli are also believed to hold a very close chemical relation to chromatin.

It is uncertain whether or not the chromosomes lose their organic identity in the daughter nuclei. Investigations on this problem are surrounded by many difficulties. It has been claimed by Guignard ('99) for *Naias* and Strasburger (:00) for several forms that the chromosomes may be followed with certainty through the period between the first and second mitosis in the spore mother cell. But other investigators have not been able to trace the chromosomes after telophase and are inclined to believe that the chromosome completely loses its identity in the resting nucleus. One of the last investigations of *Lilium* (Mottier, :03) argues strongly for the latter view, and all who have followed nuclei from one mitosis into another know that the resting nucleus with its linin network and the granular chromatin present conditions that generally make the recognition of chromosomes impossible with the instruments and technique at our command, but this does not prove that they may not be present.

The theory of the permanence of the chromosome has met with much favor because it is argued that otherwise how could the number be maintained so regularly through immense numbers of mitoses. But it can hardly be said that the doctrine is established. It has also found favor because all the events of

mitosis emphasize the importance of the chromosomes which are really the only enduring structures in the nucleus and have led to their being considered as the probable bearers of hereditary qualities.

3. The Dynamics of Nuclear Division.

Mitotic phenomena in certain plant cells present evidence that has very direct bearing on some of the theories that deal with mechanical and dynamical explanations of nuclear division. The methods of spindle formation and the various forms of kinoplasmic structures (centrosomes, centrospheres and kinoplasmic caps) which generally in plants seem not to be permanent organs of the cells all tend to support Strasburger's conception of kinoplasm, which is an outgrowth and application to plants of Boveri's well known theory of archoplasm.

The centrosome theory is supported by very few investigations in Botany, the most notable being that of Swingle ('97), for *Stypocaulon*, who believes that the centrosome divides with the aster and is maintained as a permanent organ throughout successive cell divisions. Other examples of similar conditions may be found among the thallophytes which, after all, have received very little attention, and such types as *Dictyota* and the diatoms offer excellent subjects for studies covering a series of cell divisions. But in contrast to *Stypocaulon* it should be noted that the conspicuous centrospheres of *Fucus* and *Corallina* disappear with each mitosis to be formed anew, and the same conditions obtain in the germinating spores of liverworts (*Pellia*). There seems to be no place for the centrosome in spindle formation as presented in the spore mother cells of all groups above the thallophytes (see Sec. III). Neither does mitosis in the vegetative tissues of these groups, characterized as it is by the presence of kinoplasmic caps, conform to the program of the centrosome theory.

The morphological manifestations of kinoplasm are so various that we are driven to a very general conception of its organization. Kinoplasm runs through cycles in which the structure passes from a granular condition to a fibrillar and then back again

to the granular state. By the granular state we mean one in which no fibrillæ seem to be present, but instead the microsomata are densely and homogeneously massed. It is possible that such microsomata form a closely packed network, but no such structure is visible under the microscope. The first appearance of kinoplasm at prophase of mitosis is frequently the granular condition. This state is illustrated by such accumulations as centrospheres and kinoplasmic caps and by the granular zone that has been reported around the nuclei of some spore mother cells.

Granular kinoplasm becomes fibrillar probably by the arrangement of the microsomata into a reticulum from which fibers extend freely into the surrounding cytoplasm. These fibers undoubtedly elongate during prophase, extending in various directions. Some press against the nuclear membrane and when this breaks down grow rapidly into the nuclear cavity. Of these a portion extend from pole to pole and form the central spindle. Others attach themselves to the chromosomes and lie either among the central fibers or somewhat outside of the spindle (mantle fibers). Still others may extend freely into the cytoplasm as astral rays from the pole of the spindle, a very common condition when centrosomes or centrospheres are present. A contraction of the fibrillæ, beginning with metaphase, is just as characteristic of mitosis as their elongation during prophase. The fibers attached to the chromosomes draw the latter to the poles of the spindle. The central fibers in higher plants draw away from the poles and give their substance to the cell plate. The substance of contracted mantle fibers, with other kinoplasm at the poles of the spindle, probably become distributed around the group of daughter chromosomes so that they finally lie surrounded by a sphere of kinoplasm.

It does not seem as if we knew much more about the structure and activities of kinoplasm during mitosis than is indicated in this cycle of change from a granular condition through a fibrillar state back to the granular condition, with a period when the fibers elongate and another when they contract. This with few exceptions is the history for every mitosis. The exceptions deal with peculiar conditions or structures. Thus, for example,

the astral rays of the centrospheres in the ascus instead of contracting to a center or disappearing in the cytoplasm after the last mitosis grow around the nucleus and cut out a portion of the cytoplasm to form the spores, thus contributing their substance to a plasma membrane.

There is little doubt that kinoplasmic fibrillæ actually exist as structural elements in the protoplasm. Their growth and movement in the cytoplasm and nuclear cavity, their multiplication and shifting arrangements as the spindle develops, and their contraction to the poles of the spindle or to a cell plate give these fibers an individuality that cannot be explained on the theory that they merely represent lines of force or paths of dynamic stimuli. They apparently perform all the activities mentioned above by virtue of their own structural organization which is that of rows of microsomata and in this organization resemble and are probably closely related to cilia. There is an excellent discussion of this subject by Allen, :03, p. 302, etc.

Some authors believe that there is a streaming movement in the astral rays (Chamberlain, :03, for *Pellia*) either towards or away from the pole of the spindle. This view is founded on the granular appearance of the radiations which are sometimes very thick in *Pellia* and enlarge at the points where they join the centrospheres or the outer plasma membrane. It is not altogether clear that the larger of these structures are quite the same as spindle fibers since they seem to be actually strands of cytoplasm rather than fibrillæ.

It is probably safe to assume that the forms which kinoplasm takes have relation to dynamic activities, but it is not easy to define these. Thus centrosomes, centrospheres and kinoplasmic caps may well be the centers from which dynamic stimuli extend, and they may be the focal points of other energies. These problems have been very little investigated among plants. It is obvious that differentiated regions of kinoplasm have important physical relations to other portions of the protoplasm, one of the most important being the anchorage which they give to fibrillæ, thereby largely governing the direction of such strains as come about through the contraction of these structures in the later periods of mitosis.

But the essential characteristics of kinoplasm stand out sharply from whatever point the phenomena of mitosis is viewed, and in this protoplasm with its power of forming contractile fibers is vested some of the most conspicuous activities of nuclear division as well as the important powers given plasma membranes in relation to the segmentation of protoplasm to be considered presently.

The dynamic activities concerned with the spindle present only half the story of mitosis. The other important events occur inside of the nucleus. One of these is the dissolution of a portion or the whole of the nucleolus which takes place as the spindle develops and we have already given the views of Strasburger ('95 and :00), supported by the studies of other investigators, that its substance in certain instances furnishes material for the development of the spindle. But the chief events in the interior of the nucleus deal with the accumulation of chromatin on the spirem thread which with the disappearance of the linin indicates that the latter substance may become converted into the former. The splitting of the spirem ribbon longitudinally is of the utmost significance for thereby is made possible an exact and homogeneous distribution of the chromatic material in the nucleus. We do not know how the spirem ribbon splits nor have we as yet any evidence of the origin and evolution of this peculiar activity.

(b) Segmentation of the Protoplasm.

Mitosis in the uninucleate cells of plants is generally followed by immediate cell division, which takes place in groups above the thallophytes through the formation and *cleavage of the cell plate* in the equatorial region of the spindle between the daughter nuclei. Among thallophytes, as so far studied, cell division is chiefly through *cleavage by constriction*. There are forms among the thallophytes and also in the spermatophytes whose nuclei gather about themselves a portion of the cytoplasm, wherein they lie, which becomes cut out of the general mass by a cell wall. This is *free cell formation*.

Multinucleate masses of protoplasm, such as plasmodia and

portions of cœnocytes, generally divide extensively at reproductive periods and always through *cleavage by constriction* with, however, the frequent coöperation of vacuoles which help to cut the protoplasm in the same manner as the cleavage furrows. *Cleavage by constriction* is undoubtedly the most primitive type; *free cell formation* and *cleavage by cell plates* being special and very highly developed protoplasmic activities.

1. Cleavage by Constriction.

A simple example of *cleavage by constriction* is presented by such an alga as *Cladophora*. The process consists in the building out of a ring of cellulose from the side wall into the cell cavity. The outer plasma membrane forms a fold, thus placing the two surfaces opposite one another (see Fig. 8 *a*), and the wall is laid down between these. *Spirogyra* forms its wall in precisely the same manner as *Cladophora* with this peculiarity, that the new wall finally cuts through the protoplasmic strands that connect the daughter nuclei. These strands are said to contain spindle fibers (Van Wisselingh, : 02) which may contribute to the plasma membranes forming the cell wall, as it is completed. Another illustration of *cleavage by constriction* is presented in the formation of gametes of moulds (*Sporodinia*) and the abstriction of conidia (*Erysipheæ*), both processes having been studied by Harper, '99, p. 506. In these cases a cleavage furrow proceeds from the surface inward and divides the protoplasm. The partition wall of cellulose is formed later between the two free plasma surfaces. The only differences between the processes above described are that in the first forms the cleavage proceeds more slowly and the wall follows the furrow as it progresses in the interior of the cell, while in the latter types cleavage is complete before the plasma membranes develop the wall. Cell division in the red Algæ (*Rhodophyceæ*) is also a process of constriction similar to *Cladophora*, but the wall is not generally formed entirely across the filament so that adjacent cells remain connected by thick strands of protoplasm.

These processes become much more complicated when large masses of multinucleate protoplasm are divided up into many

smaller bodies as during spore formation among the Myxomycetes and Mucorales. Very complete studies have been made of these conditions by Harper, '99 and :00a. In the slime mould (*Fuligo*) cleavage begins by furrows on the external surface which "cut down at all angles into the homogeneous protoplasm." The direction of the cleavage furrows is further complicated by the fact that many of them start from the bottom and sides of deep folds. All of the furrows may bend and secondary cleavage planes strike off from them which in time unite with one another until the protoplasm is divided progressively into very many small masses (see Fig. 8 *b*) that finally round themselves off and secrete walls, becoming spores, sometimes with one nucleus and sometimes with several.

Cleavage in the sporangium of *Synchytrium* and the moulds, as described by Harper, '99, is in general similar to that in the plasmodium with, however, the additional feature that lines or planes of vacuoles are often utilized to assist a cleavage furrow in effecting the segmentation of the protoplasm. The separation of the spore plasm of the sporangium of *Pilobolus* from the filament below begins with a cleavage furrow from the exterior; but this furrow follows and makes use of a curved plane of flattened vacuoles with the result that a dome shaped cleft is developed and two plasma membranes are presented face to face, which form the columella wall between them. The segmentation of the spore plasm in *Pilobolus* is affected somewhat similarly through the coöperation of cleavage furrows from the exterior with vacuoles which cut into the protoplasm at various angles to meet one another and the cleavage furrows. The bodies first formed in the sporangium of *Pilobolus* are not the final spores. Harper suggests that they may correspond to the zoöspores of *Saprolegnia*. They are generally uninucleate and begin immediately a period of growth within the sporangium characterized by extensive nuclear multiplication and several divisions of the protoplasmic body by constriction.

Harper finds that the spore plasm of *Sporodinia* is separated from the filament below by a dome-shaped plane of flattened vacuoles which fuse together and, unlike *Pilobolus*, cut their way to the surface of the sporangium. Thus the cleavage is deter-

mined entirely by the activity of vacuoles. Spore formation, however, is accomplished by cleavage furrows which progress from the exterior inwards and, without the aid of conspicuous vacuoles, cut out multinucleate masses of protoplasm which become the spores.

Dean Swingle (:03) has extended the studies of Harper on spore formation in the molds to *Rhizopus* and *Phycomyces*. He confirms Harper's account of the general processes of cleavage by furrows coöperating with vacuoles, and notes the following characteristics in the types studied. In *Rhizopus* the position of the columella is determined by a dome-shaped series of flattened vacuoles which fuse and meet a cleft that extends upward from the outer plasma membrane at the base of the sporangium. The spores are formed in *Rhizopus* by branching systems of curved furrows that cut the protoplasm into multinucleate masses, and in *Phycomyces* by angular vacuoles that develop into furrows which extend in various directions and unite with one another and with clefts from the region of the columella.

Other excellent illustrations of *cleavage by constriction* are presented in the sporangia of such types as *Hydrodictyon*, *Cladophora* and *Saprolegnia*. Timberlake (:02) has given an account of *Hydrodictyon*, and the events are also fairly well understood for *Saprolegnia*. Segmentation begins in *Hydrodictyon* by the development of cleavage furrows in the outer plasma membrane, which cut into the protoplasmic layer at right angles to the surface and meet similar furrows that make their way from the large central vacuole outward. These cleavage planes spread laterally, uniting with one another, until the protoplasm is all divided into uninucleate masses which become the zoöspores (Fig. 8 c). In *Saprolegnia* (see Davis, :03, for general account) conspicuous cleavage furrows develop from the central vacuole and make their way to the exterior, finally breaking through the outer plasma membrane. When this takes place there is an immediate escape of cell sap, which was under pressure, and a shrinkage of the sporangium so that the zoöspore origins appear to fuse, but this is not really the case, for cleavage is continued and the zoöspores soon separate.

A physiological explanation of *cleavage by constriction* must

consider two sets of factors. There is an evident contraction of the protoplasm in many examples because water is given off. The shrinkage of the surface would undoubtedly form furrows, but, as Harper has pointed out, these furrows do not develop in an accidental manner. Non-nucleated masses of protoplasm are never separated from the nucleated, but the segmentation proceeds after a system by which the final products contain only one nucleus or at most a limited number. So it is probable that the nuclei are the ultimate centers controlling the segmentation which at its commencement may be quite irregular. This explanation of sporogenesis in the plasmodium and the sporangium is not altogether satisfactory for the cell division of *Cladophora*, the abstriction of conidia or the development of the gametes of a mould. In these examples the cleavage begins at definite regions of the plasma membrane, so that the stimulus must be local, and the direction of the plane has a definite relation to the axis of the plant.

It is important to note (see Harper, :00, p. 240-249) how inadequate are some of the well-known theories of the segmentation of protoplasm as explanations of *cleavage by constriction*. Hofmeister's law ('67) that cell division is across the axis of growth obviously cannot be applied to the irregular segmentation in the plasmodium and sporangium, nor is Sachs' well-known law of growth in vegetative points adequate. Sachs, '94, and in the *Lectures on the Physiology of Plants*, chap. XXVII, conceives a growing point of a higher plant or an embryonic structure as a mass of protoplasm whose cell walls are determined by principles of rectangular intersection of perpendicular planes. The outer form of the structure determines the angles of periclinal and anticlinal walls and the transversals conform to these. There is not the slightest hint of such an order in the distribution of cleavage planes in the multinucleate masses of protoplasm just described and Sachs' law in so far fails of general application whether or not it be satisfactory for the conditions with which he especially deals. There are also explanations of cell division, applicable to the tissues of many higher organisms, based on the position of the nuclear figure in the cell, which determines the position of the cell plate but these theories cannot handle the events in the

plasmodium or sporangium where the cleavage planes are formed without regard to the time of nuclear division or the position of mitotic figures.

2. Cleavage by Cell Plates.

Cleavage of the protoplasm by means of the cell plate is almost universal in cell division of plants above the thallophytes. It is one of the peculiarities of plant cells, having been found in comparatively few animals and there represented rather imperfectly by the so-called mid-body. The general events of the process have been known since Treubs' studies of 1878, and were clearly described by Strasburger in 1880. Timberlake, :00, in a recent paper gives an historical review of the subject.

When, after the metaphase of mitosis, the two sets of daughter chromosomes separate from one another there is left between them the spindle, made up of the central fibers. The first appearance of the cell plate is a line of granules in the equatorial region of this spindle where the nuclear plate formerly lay. But several important events proceed this condition. The connecting central fibers begin to thicken, first near the daughter nuclei, and then gradually towards the equatorial region of the spindle. The number of fibers may increase greatly, probably by the separation of bundles of fibrillæ composing the spindle into independent elements (Timberlake, :00, p. 94). But there is evidence that new fibrillæ are sometimes formed from the vicinity of the daughter nuclei, some of which may enter the spindle and coöperate with the connecting fibers. In certain forms (*e. g.* Allium) there is an accumulation of a stainable substance between the connecting fibers in the equatorial region of the spindle. The reaction of this substance to stains indicates a carbohydrate composition.

The cell plate really begins with the thickening of the connecting fibers in the equatorial plane of the spindle. In some forms these thickenings are elongated bodies, in others mere granules. The earlier writers (Treub, '78, Zacharias, '88) did not believe that they came from the spindle fibers, but there seems to be now no doubt of their origin from these elements,

which contract and thicken as the plate develops. The bodies composing the cell plate finally lie in a plane extending the entire width of the spindle (Fig. 8 *d*) and they then broaden and come in contact with one another to form a continuous membrane, which, as has been said, may lie in a matrix of carbohydrate material. The cell plate grows rapidly as the central spindle fibers shorten and contribute their substance to the structure. During this contraction the surrounding cytoplasm

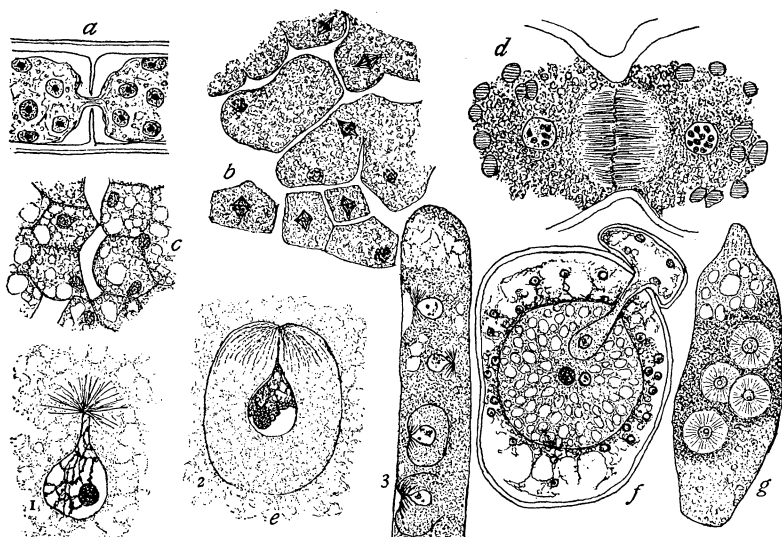


FIG. 8.—Segmentation of the Protoplasm. *a, b, c*, cleavage by constriction. *d*, cleavage by cell plate. *e, f, g*, free Cell Formation. *a*, cell division in *Cladophora*. *b*, cleavage of spore plas in *Fuligo*. *c*, spore formation in *Hydrodictyon*. *d*, first division of spore mother-cell in *Pellia*. *e*, spore formation in ascus, 1 and 2 (*Erysiphæ*) astral fibers cutting out cytoplasm around nuclei, 3 portion of ascus with developing spores (*Lachnea*). *f*, oögonium of *Albugo*, egg surrounded by membrane pierced by antheridial tube, cenocentrum and female gamete nucleus within. *g*, egg of *Ephedra* with four embryo cells. After Strasburger Harper and Timberlake.

enters the region between the barrel shaped group of fibers and the daughter nuclei (Fig. 8 *d*). It is probable that the cell plate is composed entirely of the substance of spindle fibers and in consequence is kinoplasmic in character. The cell plate widens with the accretion of material from the central spindle, which in some cases is assisted by the radiating fibers that, lying outside of the spindle, contract and add their material to the edge of the plate. The cell plate thus extends laterally and finally reaches

the neighboring cell walls, fusing with the outer plasma membrane. There are certain mitoses, as in some spore mother cells and in the embryo sac (see Section III) where the cell plates are absorbed into the cytoplasm leaving the original cell with two or more nuclei and without partition walls. It is uncertain whether the edge of the plate is ever extended by the development of additional peripheral fibrillæ (Timberlake, :00, p. 161) from the daughter nuclei.

Cell division is accomplished by the splitting of the cell plate (Strasburger, '98) into two plasma membranes. The division generally begins in the center and the cleft progresses towards the periphery until it reaches the cell wall. During the process the thickened rod shaped portions of the spindle fibers are pulled apart. There are thus left two kinoplasmic membranes opposite one another and continuous with the outer plasma membrane surrounding the daughter cells. The cause of this cleavage is not apparent, but there are reasons for believing that the split is essentially a thin vacuole which, starting near the center, cuts its way through the cell plate to the periphery after a manner very similar to the behavior of vacuoles during the cleavage of the plasmodium and in the sporangia of certain moulds. And there may be shown in this activity a relationship of *cleavage by cell plate* to some of the events of *cleavage by constriction*. After division is complete there follows the formation of a cell wall between the two cell surfaces after the method usual to plasma membranes.

The new cell wall generally begins in the oldest portion of the cell plate where the cleft first appeared and is gradually built out peripherally until it reaches the side walls. The first indication of the wall is the appearance in the cleft of a stainable carbohydrate substance which resembles the material that was primarily present between the fibers of the central spindle and which disappears with the formation of the cell plate. This material is probably the basis of the first deposits on the surface of the two plasma membranes, but the nature of the final substance is exceedingly various. A cell wall may be formed that is homogeneous throughout but often the thickened wall presents three regions, two layers of a cellulose basis formed by the

respective plasma membranes and between them the so-called middle lamella.

The middle lamella has been the subject of much discussion. It is not the remains of the cell plate as was once supposed. Neither is it exactly a cement between two cell walls. Its history is undoubtedly various, for the composition shows much plasticity. The origin of the middle lamella at the surface of a plasma membrane indicates a morphology similar to a cell wall, but the substance, pectic in character, shows transformations far removed from the cellulose compounds that are formed later and which give thickness to the cell wall. Allen (:01) discusses the subject in detail.

The origin of the cell plate is a subject of interest which will be further discussed in Section VI. There are some types, especially among the thallophytes, where a cell plate is present, but apparently in a somewhat undeveloped and rudimentary condition. These forms suggest transitional conditions between *cleavage by constriction with the aid of vacuoles*, so general among the thallophytes, and *cleavage by the cell plate*, characteristic of higher groups. The most interesting examples are Anthoceros, Chara, Basidiobolus, Pelvetia, Fucus, and Sphacelaria.

Cell plates are formed with each of the two successive mitoses in the spore mother cell of Anthoceros (Van Hook, :00; Davis, :01, p. 158), but the structure in some species is exceedingly small (*e. g.*, *A. lævis*) and can scarcely extend more than one-tenth of the distance across the cell. It is larger in other forms, as in the one studied by Van Hook; but even there the nuclear figure of the second mitosis is only one-third of the width of the cell. The protoplasm divides simultaneously in the four spores with the characteristic arrangement. If this division were determined entirely by cell plates there would be required an extensive development of fibrillæ, of which there is no evidence in the cell. But their place seems to be taken by numerous delicate strands of cytoplasm which connect the four protoplasmic masses, each of which contains a large chromatophore and an accompanying nucleus. A film is formed in the intermediate region, and this marks the position of the cell wall. It is, of course, quite certain that the two cell plates of the second mitosis are

a part of this membrane and may start its development, but the final structure must contain very much more material than could possibly be contributed by the sparsely developed spindle fibers. Thus, although the splitting of the cell plate may start the process of segmentation, its final course and end is probably determined by cleavage through vacuoles, thus utilizing a method characteristic of the thallophytes.

Chara appears to have a fairly well developed cell plate (Deb-ski, '97) which extends almost entirely across the cell, presenting very exceptional conditions among the thallophytes. This peculiarity is in keeping with other characters of the spindle, which begins its development outside of the nuclear membrane and, lacking centrosomes, resembles the nuclear figures of higher plants. It is possible that nuclear studies upon *Chara* throughout ontogeny might show a variation that would be very significant for the evolutionary problems concerned with the structure of protoplasm.

Fairchild ('97) reports a cell plate for *Basidiobolus* when the beak cells are cut off from the gametes. The structure, as figured and described, is not, however, conspicuous. He points out general resemblances between cell division in this form and in the *Conjugales*, where, as Van Wisselingh (:02) described later for *Spirogyra*, spindle fibers connect the daughter nuclei and may coöperate towards the end of cell division with a cleavage furrow from the side of the cell.

The conditions in the *Fucales* are not altogether clear. Both Strasburger ('97a) and Farmer and Williams ('98) report that the central spindle disappears in *Fucus* without the formation of the cell plate and that the wall is developed between the daughter nuclei in a region of granular cytoplasm. However, in *Pelvetia* some of the radiating fibrillæ from opposite sides of the daughter nuclei bend around these structures and end in the new wall. It is not plain that they contribute much if anything to its formation in the way of substance, but it would seem probable that they hold a directive relation to the structure (Farmer and Williams, '98).

The *Sphacelariaceæ* seem to be somewhat similar to the *Fucales* in their methods of cell division. The beautiful figures of

Swingle ('97) for *Stypocaulon* give details of the region of the cytoplasm that forms the partition wall between the daughter nuclei. There is a zone of fine meshed protoplasm between much larger vacuoles. It is possible that some very long fibrillæ may connect the daughter nuclei with this zone, but they do not form a cell plate. Consequently the wall must be developed in this delicate alveolar layer, which probably splits along some plane of vacuoles. The process of cleavage is then really related to such activities of vacuoles as occur in the sporangium of the *Mucorales* and in the plasmodium. But the position of the alveolar layer may be determined by the fibrillæ, since it is always situated nearest to the smaller of the two daughter nuclei.

It seems likely that the process of cleavage in the *Fucales* will be found to be similar to *Stypocaulon* when the details of structure in the internuclear cytoplasm is known. So this group, with others, is likely to furnish conditions in which spindle fibers may determine the position of the cell wall and exert a directive influence upon it without actually laying down a cell plate. As has been pointed out, the splitting of the cell plate is probably a cleavage along a very thin flat vacuole, so that the process in its essential characters is the same as cleavage through a series of vacuoles. Thus *cleavage by the cell plate* is possibly an outgrowth from that phase of *cleavage by constriction* in which the extensive fusion of vacuoles determines the planes of separation. The important advance lies in the new factors, introduced through the activities of fibrillæ, which become very conspicuous as actual contributors of material to the kinoplasmic film which is laid down as the cell plate. This function of the fibrillæ probably developed slowly from conditions such as those in *Stypocaulon* and *Pelvetia*, where their influence upon the position of the cell wall, if any at all, can scarcely be more than directive.

3. Free Cell Formation.

Whenever a nucleus becomes the center around which cytoplasm is gathered and separated from the rest of the cell contents, so that the new cell lies freely in the protoplasm of the old, this is *free cell formation*. Illustrations are presented by

the spores of an ascus, the oöspore of the Peronosporales, the embryo cells of Ephedra, and probably other gymnosperms, and in some cases seemed to be exemplified in the conditions presented by the egg and synergids and the antipodals of the embryo sac.

Spore formation in the ascus is known through the studies of Harper ('97 and '99). After the final divisions in the ascus the nuclei lie in the cytoplasm, each with an aster at its side (Fig. 8 *e*, 3). A delicate prolongation carries the aster with its centrosphere away from the main body of the nucleus (*e*, 1). The rays of the aster now bend over and grow around the nucleus, presenting an umbrella-like figure (*e*, 2). They finally meet on the opposite side, and thereby cut out a portion of the cytoplasm which is included in the spore. The substance of the aster fibers forms the basis of a kinoplasmic film which becomes the plasma membrane of the ascospore and develops the spore wall externally after the usual method. This peculiar activity of an aster is unparalleled in plant or animal cells.

Oögenesis in the Peronosporales has been described in some detail by several authors, but the process has not generally been called *free cell formation*. Yet at the end of the process the oöspore, enveloped by periplasm, lies free in the oögonium. In the beginning the oöplasm gathers in the center of the oögonium as a denser alveolar region around that peculiar protoplasmic body (generally present) the cönocentrum. This accumulation forces the vacuoles, together with most of the nuclei, to the periphery, where they lie in a sort of protoplasmic froth next the cell wall and constitute the periplasm. The spore wall develops at the boundary of the oöplasm, so that it lies close to the large vacuoles (Fig. 8 *f*) in the periplasm. There must be an accumulation of kinoplasm, perhaps from the plasma membranes of numerous vacuoles, to form a delicate layer between the two regions of the oögonium. This layer of kinoplasm probably splits along the line of vacuoles between the oöplasm and periplasm, for the primary walls are certainly established between two plasma membranes, because the secondary layers are added to it from both sides. Nuclei in division frequently lie very close to the boundary of the oöplasm, but there is no evidence

that the kinoplasmic membrane has any relation to these mitotic figures. That is to say, there are no fibrillæ to contribute substance to the membrane, and its development must be concerned with vacuoles alone. In this respect the process recalls the part played by vacuoles in the plasmodium and in certain sporangia during cleavage by constriction.

Free cell formation after the method in the egg of *Ephedra* (Strasburger, '79), which is also likely to be found among other gymnosperms, takes place during the differentiation of the embryo cells. The cytoplasm collects around each nucleus, forming a sphere (Fig. 8 *g*), and a wall is developed on the outside of this body. Details of the process are not known, and it is not clear whether the position of the membrane is determined by the vacuoles that must border upon this region or whether there are fibers radiating from the nucleus which might lay down a cell plate around the denser protoplasm; but the evidence favors the former possibility.

Somewhat similar conditions are presented in the egg apparatus of many embryo sacs. In certain forms (*e. g.*, the lily so well described by Mottier, '98) the egg nucleus and synergids are thickly invested by radiating fibers, and these, together with the cell plates, may readily determine the position of the plasma membrane that forms the cell wall. But fibers do not seem to be conspicuously present in the egg apparatus of many other embryo sacs (Excellent illustrations can be found among the *Ranunculaceæ*). In these cases the protoplasm collects around the nuclei as dense areas bordered by vacuolar cytoplasm, and it is possible that the vacuoles by fusing with one another cut out these respective regions and thus determine the plasma membranes of the egg and synergids. Such processes would extend the activities of vacuoles, which accompany cleavage by constriction in the thallophytes, to the highest groups of plants.

It is curious that with all of the work upon the embryo sac we should know less about the segmentation of the protoplasm around the synergid, antipodal, and segmentation nuclei in this structure than in the sporangia of the molds, the ascus, or during spore formation in the *Myxomycetes*.

(*To be continued*).

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